REFERENCE MANUAL

AO Reichert Model 975C and 976C HISTOSTAT™ Cryostat Microtome

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AO Reichert™

AO Reichert Scientific Instruments
Division of Warner-Lambert Technologies, Inc.
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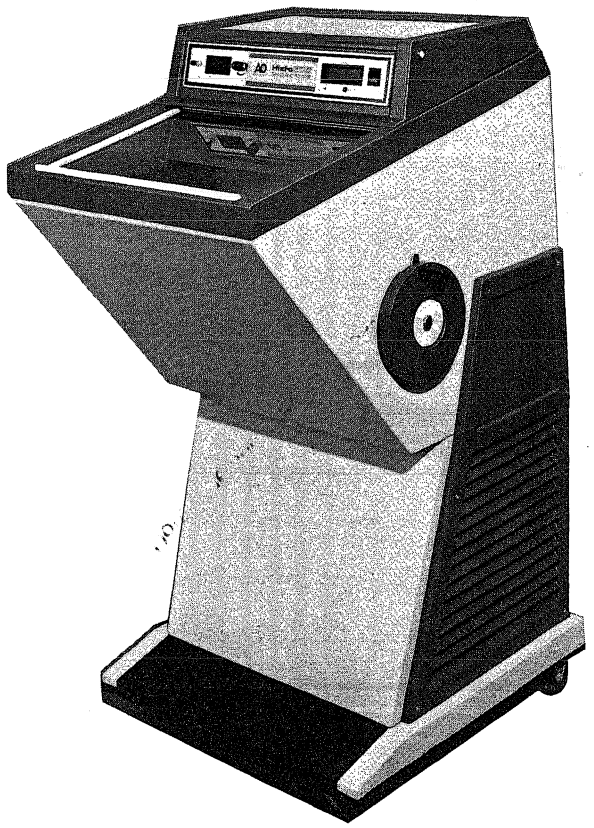
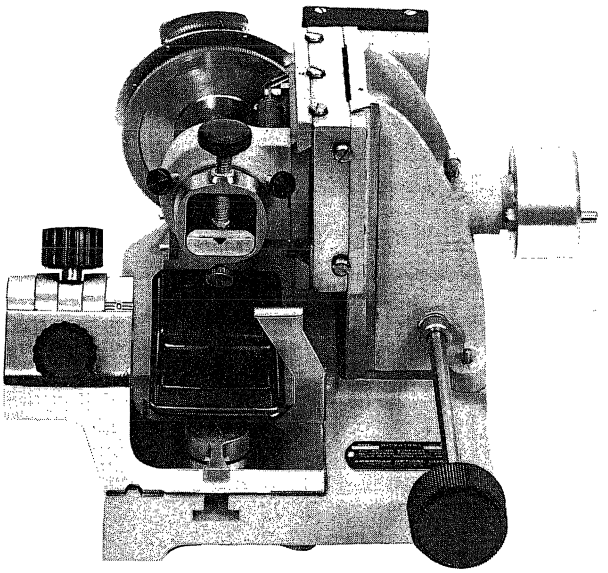




Model 975C: 115 volts, single phase, 60 Hz
Model 976C: 220 volts, single phase, 50 Hz

Figure 1. AO Reichert HISTOSTAT Cryostat Microtome

INTRODUCTION

You now possess an AO Reichert instrument of uncompromising quality that will provide you with many years of precise, satisfying service. This Manual has been written to help insure that you secure optimum performance from your AO Reichert HISTOSTAT Microtome. It describes initial set-up procedure, operation, care and maintenance.

The rotary microtome, because of its weight, is removed from the cabinet and packaged separately for shipment to properly protect its precision mechanism. The cabinet and microtome are self-aligning and the microtome can be quickly and easily installed. If additional copies of this instruction manual are desired to aid in training new personnel, or for other purposes, they are available, at no charge, as an AO Reichert customer service. For copies of Reference Manual 975C-101 write: Sales Department, AO Reichert Scientific Instruments, Instrument Division, Box 123, Buffalo, NY 14240.

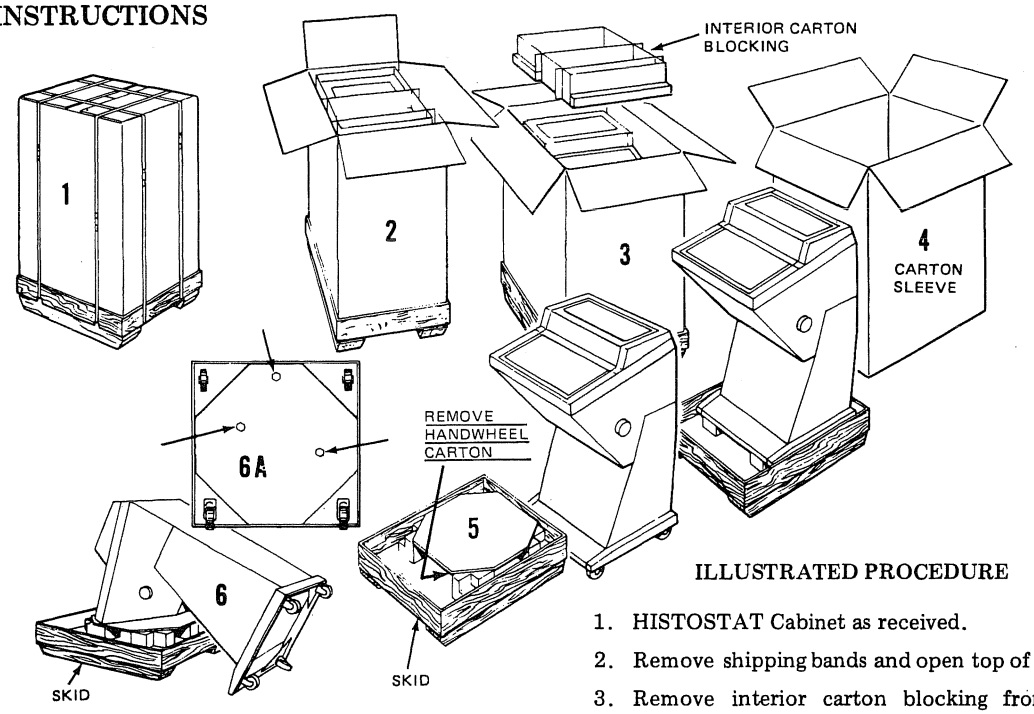
The AO Reichert Model 975C HISTOSTAT Cryostat Microtome consists of:

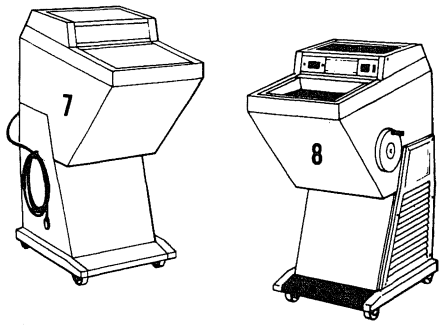
855 Cabinet
840 Microtome
831 Knife Holder
832 Anti-Roll Guide
846 Heat Extractor
942 Microtome Knife (120 mm)
833 Object Disc (3 supplied)
970 Lubricant

SET-UP PROCEDURE

PRELIMINARY STEPS

UNPACKING INSTRUCTIONS





ILLUSTRATED PROCEDURE

1. HISTOSTAT Cabinet as received.
2. Remove shipping bands and open top of carton.
3. Remove interior carton blocking from top.
4. Lift carton sleeve and plastic bag from Cabinet.
5. Lift Cabinet clear of skid (two people required).
6. Place skid IN FRONT OF CABINET and tip Cabinet forward onto skid, as shown.

6A. Bottom of Cabinet is now exposed. Remove the THREE RED-PAINTED SHIPPING BOLTS ONLY. (Reserve these bolts, as well as washers, for future transporting, if required.)

7. Return Cabinet to upright position. Remove Handwheel Assembly Carton from its packed position on skid.
8. Remove wire ties from Power Cord. Attach Handwheel.

TO ATTACH HANDWHEEL

1. Remove screw in center of shaft.
2. Remove tape holding keyway on shaft.
3. Hold end of shaft inside cabinet and slide handwheel on shaft, aligning keyway slot on handwheel to keyway on shaft.
4. Place metal disc in recess on face of handwheel.
5. Secure disc and handwheel to shaft with screw.

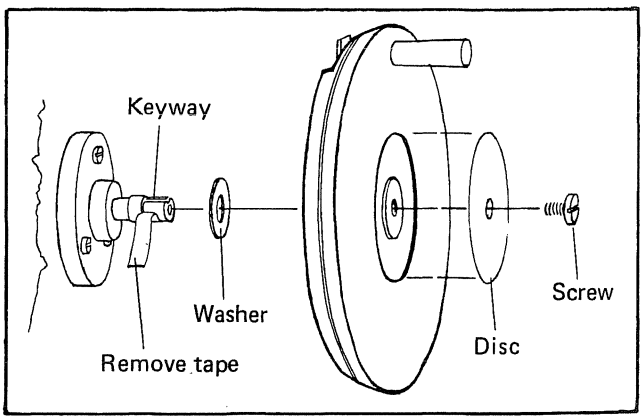


Figure 2

MICROTOME

1. Remove all shipping supports and unpack the microtome and accessories.

NOTE: Check to see that shipment is complete.

2. Lubricate the vertical and horizontal slideways of the microtome (see Figure 6) with the special lubricant provided.

INSTALLING MICROTOME IN CABINET

CAUTION: The walls of the HISTOSTAT cabinet have been fabricated to a high quality finish. Exercise extreme caution when installing the heavy 840 Microtome. It is advisable to remove the glass door during installation. See "NOTE" on page 11.

1. Set the microtome down into the cabinet so that it rests against the guide bar, A. Figure 3.

2. Slide the microtome to the right as far as possible. The drive coupling, A, Figure 4, of the microtome will seat against the cabinet drive hub, B, Figure 4.

3. While holding the microtome in position (to prevent tipping backward), place the hold-down bolt thru the hole in the front of the microtome base and fasten into cabinet hole, B, Figure 3.

   Use slotted head screw supplied with microtome.

4. Turn the handwheel until the spring-loaded coupling pin engages the hub and lock handwheel.

5. Attach the catch tray, A, Figure 5, by positioning back edge of tray under holding screw B, Figure 5. Push front of tray down to secure in position.

6. Read the section entitled "Knife Holder" under general heading "USE" then attach knife to knife holder and knife holder to microtome.

7. Lay the brush holder, C, Figure 5, in position as shown.

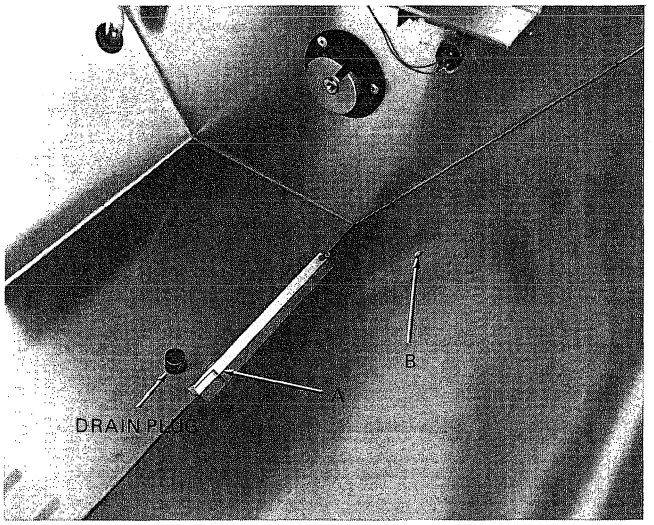


Figure 3

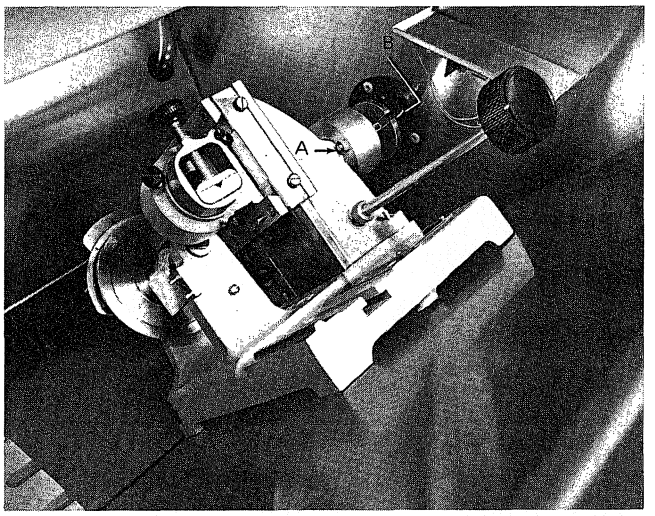


Figure 4

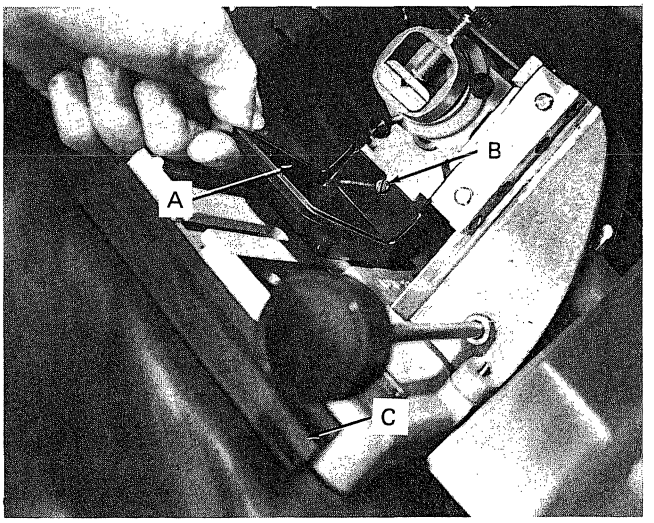
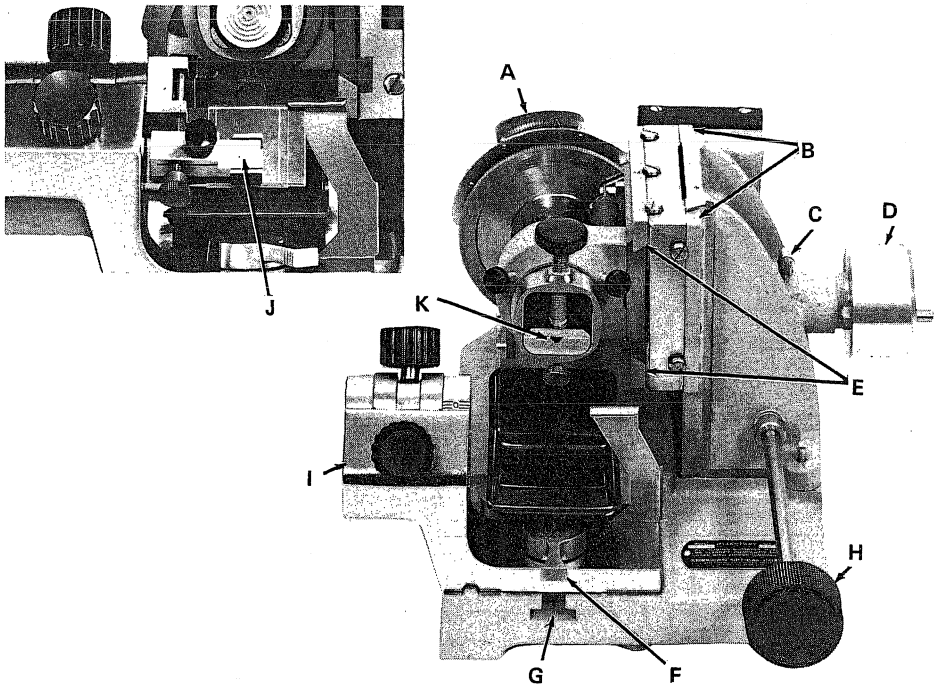


Figure 5



A) Thickness Indicator
B) Vertical Slideways
C) Oil Cup
D) Drive Coupling
E) Horizontal Slideways
F) Knife Holder Lock Lever
G) Slot for Knife Holder
H) Fast Feed Control
I) Knife Holder
J) Anti-Roll Guide
K) Object Clamp

Figure 6

SETTING CABINET CONTROLS

The HISTOSTAT cabinet has no "Power On" switch. Power is automatically applied to the instrument when the power supply cord is plugged into a grounded outlet. "Power On" is indicated by lighted digital clock display. Temperature display will not appear until "COOL" switch has been activated.

CLOCK INSTRUCTIONS

Clock Flashing: When the HISTOSTAT is initially plugged in or if power is interrupted, the digital clock will flash. Setting or resetting time will cause flashing to stop.

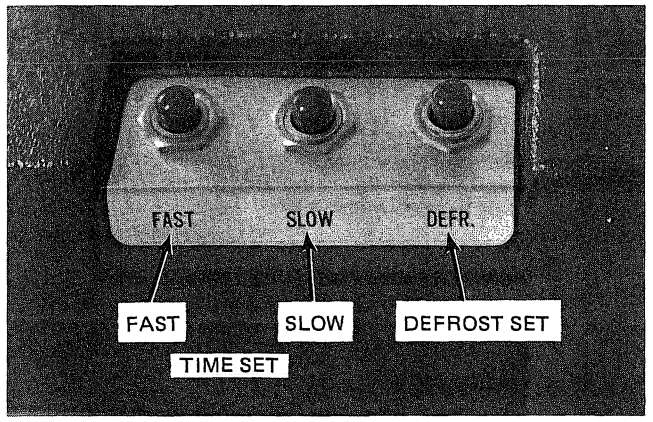


Figure 7

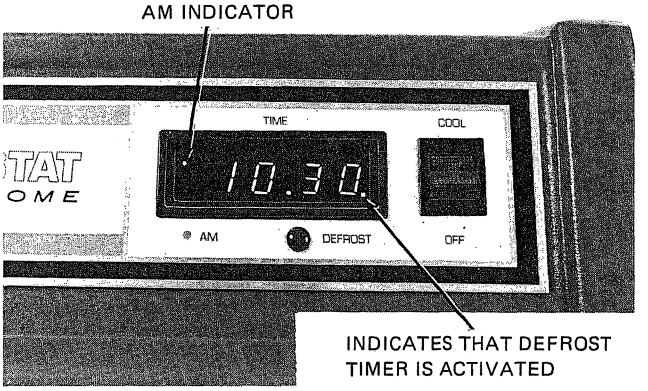


Figure 8

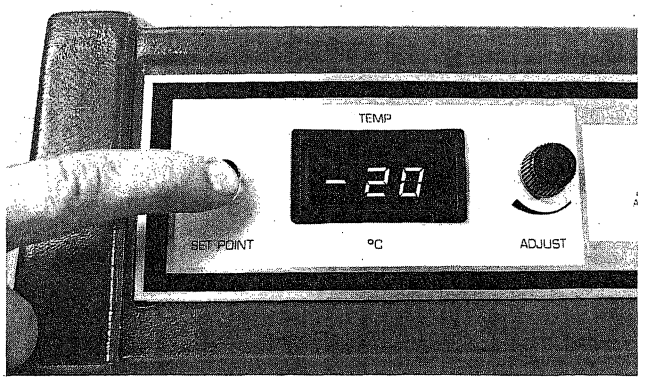


Figure 9

Setting Displayed Time

1. Press either "FAST" or "SLOW" pushbutton to advance the displayed time, Figure 7, located at rear of the cabinet.

2. Red dot in upper left corner of clock display indicates AM hours, Figure 8.

3. Simultaneously pressing both "FAST" and "SLOW" pushbuttons will set 12:00 PM on the display.

SETTING TO DESIRED TEMPERATURE

1. Turn refrigeration system on by pushing the "COOL/OFF" switch located on the right side of the control panel, to the "COOL" position, Figure 8.

WARNING: WHEN STARTING REFRIGERATION UNIT AFTER INSTRUMENT HAS BEEN OFF FOR A PROLONGED PERIOD, THE COMPRESSOR MAY SOUND UNUSUALLY LOUD. THE NOISE LEVEL SHOULD REDUCE IN 30 SECONDS. IF IT DOES NOT, STOP THE REFRIGERATION UNIT IMMEDIATELY AND NOTIFY AO TECHNICAL SERVICES.

2. Depress and hold "SET POINT" button, Figure 9.

3. Turn the "ADJUST" knob to obtain the desired operating temperature, as displayed, Figure 9.

4. Range of adjustment is from 0° to -30°C.

5. When "SET POINT" button is released, actual temperature again will be displayed.

NOTE: Due to the large mass of the cabinet and the microtome, sufficient time must be allowed before the displayed temperature truly indicates the temperature of the cabinet. Allow approximately four (4) hours for temperature to reach equilibrium at normal (75°F) room temperature and cabinet temperature setting of -25°C.

Care must be taken <u>not</u> to change the set point by inadvertently turning the "ADJUST" knob. To check the set point temperature, simply depress the "SET POINT" button.

DEFROST CYCLE

The HISTOSTAT cabinet will automatically defrost itself once every 24 hours at the preset time.

Setting Defrost Time

NOTE: It is strongly recommended that the defrost timer be set to 5 hours before the next scheduled use of the equipment.

1. Depress and hold "DEFR" button, located at right rear of the cabinet, Figure 7.

2. Press either "FAST" or "SLOW" pushbutton to advance the display to the time when defrosting must start.

3. Simultaneously pressing both "FAST" and "SLOW" pushbuttons will set 12:00 PM on the display.

4. When the "DEFR" button is released, the display will revert to real time.

5. A red dot in the lower right-hand corner of the clock display indicates that the defrost timer is on and defrosting will start at the preselected time, provided the cabinet temperature is below 10°C, Figure 8.

Defrost On

The HISTOSTAT cabinet is being defrosted when the red "DEFROST" light, located below the clock, is on.

If immediate defrost is required, simply advance the defrost time to the displayed time.

Remember to reset the defrost time back to its original setting after the defrost is completed.

IMPORTANT:

In case of clock or timer malfunction, defrosting is possible, activated manually.

For instructions, request Customer Service Manual, TSM-67-1, Section XVI, "Manual Defrost Procedure."

USE

KNIFE HOLDER (Catalog No. 831)

The knife holder has been designed specifically for HISTOSTAT Microtome work. Three simple controls make it extremely easy to use. The knife is held rigidly at only one end to greatly facilitate removal of cut section on the microscope slide. The holder slides forward and back in the slot G shown in Figure 6, and should be locked into position with lever F, flush with the front edge of the base or not further than 1/4 inch in from the edge. The knife guard as shown in Figure 10, swings out of the way for removing or installing the knife. When the knife is installed, the knife guard should be swung up into the position as shown in Figure 11, to shield the corner of the knife.

The operator should become familiar with properly inserting the knife in the holder, by removing the holder from the microtome and examining the internal locking mechanism. Refer to Figure 11. When knob A is turned, the knife is drawn up by an internal U-shaped piece called a "clevis", and wedged into the opening to hold the knife securely. When knob A is released, and **tapped downward**, the clevis lowers and the knife is released, permitting withdrawal. When inserting the knife, care must be taken to prevent tilting the corner of the knife upward to hit the screw which engages the clevis from knob A. When the knife is inserted straight, ample clearance is provided.

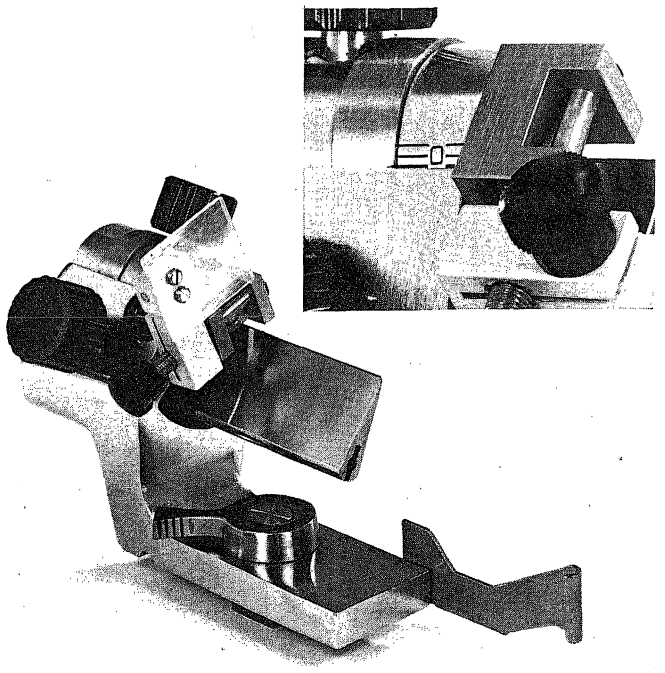


Figure 10

KNIFE

The knife holder described above is designed to accept the AO Reichert Microtome Knife No. 942, 120mm in length. A truly sharp knife with a uniform cutting facet of proper angle and width is mandatory for good results. A good microtome is often blamed for poor sectioning caused by poor knife edge or incorrect angle. Rust preventative material on a new knife should be very thoroughly removed with Xylol before installing in the knife holder.

As one end of the knife becomes dull, it can be reversed 180° to use the other half. Knife angle is accomplished by releasing knob B shown in Figure 11. An arbitrary scale of 5° increments is provided to show the degree of tilt. Position 0 (second line from the top) will give the following knife clearance angles with an AO Reichert 942 knife.

New: 30° angle = 3.6° approx.
Resharpened: 34° angle = 1.6° approx.

For the average knife and specimen, a setting to the line below 0 (third from the top) is recommended. Refer to inset, Figure 10.

IMPORTANT

The knife holder is designed to accommodate only new or factory reconditioned AO Reichert knives. Knives which have been resharpened by any method other than the AO Reichert factory process or the AO Reichert Knife Sharpener cannot be properly positioned in the knife holder.

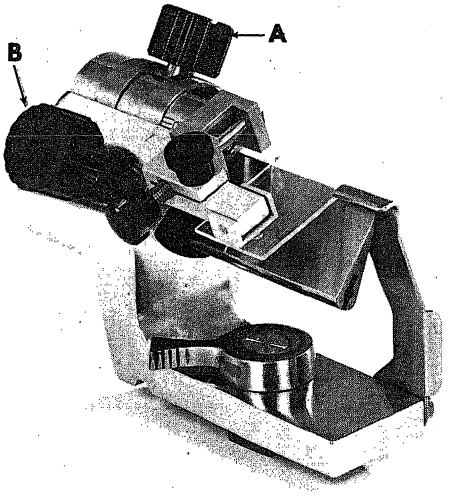


Figure 11

ANTI-ROLL GUIDE (Catalog No. 832)

The Anti-Roll Guide is shipped separately and is easily installed with Allen wrench and screw provided.

The clearance between the knife and guide should be set to approximately 30°, Figure 11.

Knob A, shown in Figure 12, provides a micrometer screw adjustment of the plate. Knob B locks this movement after correct adjustment has been achieved. The edge of the plate is adjusted to almost coincide with the knife edge and parallel with it. It should not protrude beyond the knife edge, since it must allow the knife edge to cut and immediately guide the cut section under the plate and along the knife face plate. Correct separation between the edge of the guide plate and the knife face is provided by two raised lands, C, Figure 12, 0.0635 mm high. If at any time these lands are damaged in cutting, a new plate should be installed. This is easily accomplished by removing the two screws that retain the plate. After discarding the damaged plate, install the new plate making sure that the leading edge of the plate is coincidental with the cutting edge of the knife.

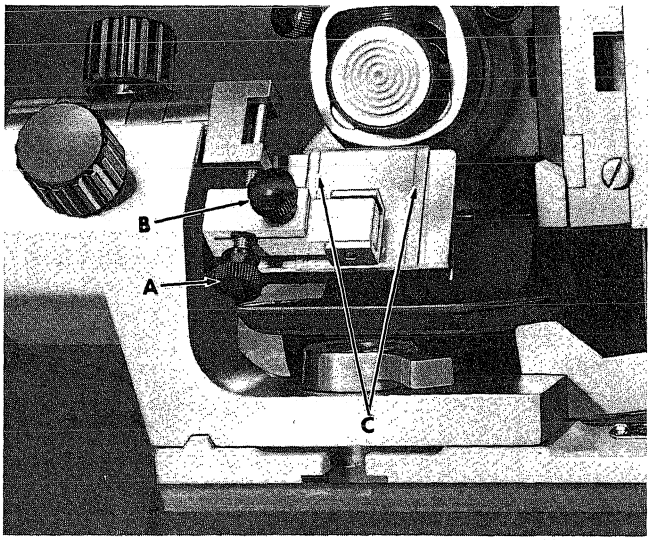


Figure 12

To Adjust

1. Turn Knob B counterclockwise to unlock screw adjustment.
2. Turn Knob A to raise (CCW) or lower (CW) the Guide's edge approximately 1 mm below the knife's edge.
3. Turn Knob B clockwise to tighten screw adjustment. Do not lock.
4. Adjust the Guide so that **both** lands, C, are in contact with the face of the knife.
5. Raise the Guide to the knife's edge.
6. Turn Knob B tightly clockwise to secure the adjustments.

GENERAL OPERATION

Mounting the object disc in the microtome, adjusting the knife, and squaring the specimen are for the most part identical to techniques used in paraffin sectioning. The essential difference is that frozen sections do not ribbon, so operation of the handwheel, instead of being a continuous motion, consists of taking a single cut. With moderate speed, the single section should spread uniformly along the knife face under the anti-roll plate. Skillful use of a camel's hair brush will be preferred for flattening some specimens, especially when using "cold slide" technique. In this case the section is teased onto a microscope slide with camel's hair brush as it comes off the knife and the anti-roll plate is not used.

HEAT EXTRACTOR (Catalog No. 846)

The copper heat extractor when placed on top of the specimen, will speed the freezing process by rapidly drawing off heat from the specimen. Before using, best results are obtained by lightly wetting the bottom of the heat extractor with HISTOSTAT Microtome lubricant. To use the heat extractor, grasp the black plastic knob, and lower the extractor onto the tissue in position on an object disc. After 30 seconds, give knob a clockwise twist and lift it from the tissue. Always work with cold object discs which have been stored in the HISTOSTAT cabinet.

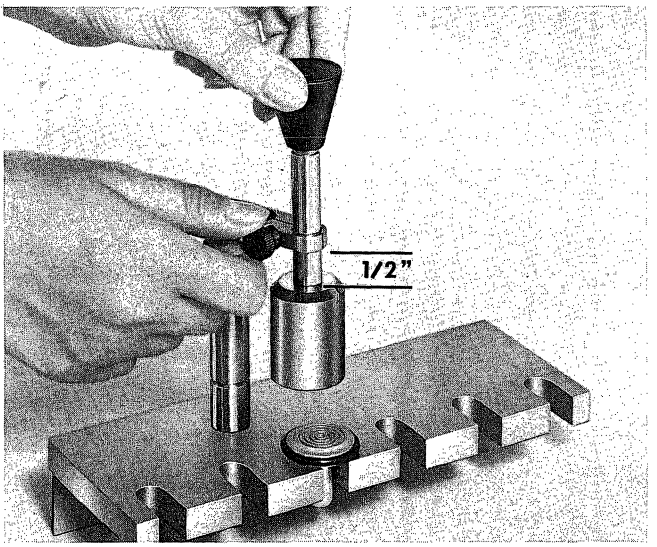


Figure 13

The weight or "load" applied on the specimen by the heat extractor can be regulated. A counter-balanced spring provides infinite adjustment to permit fast freezing without cell distortion. To adjust:

1. Loosen the set screw knob on the side of the extractor as shown in Figure 13.

2. Lift the extractor by the large top knob until, as illustrated, approximately 1/2" of the outer sleeve remains below the horizontal portion of the extractor mount. Tighten the set screw.

3. Allow the extractor to lower onto the specimen and check weight being applied.

4. If less weight is desired, raise position of sleeve in mount; lower to increase weight.

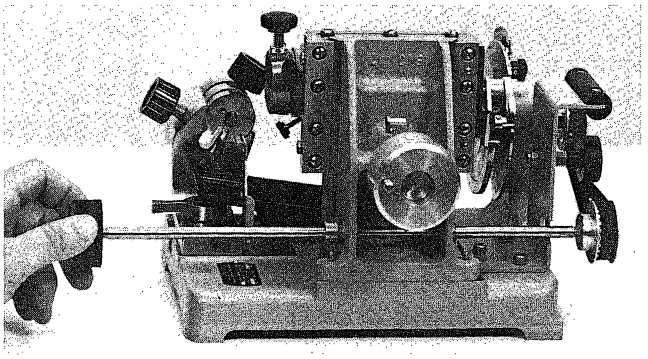


Figure 14

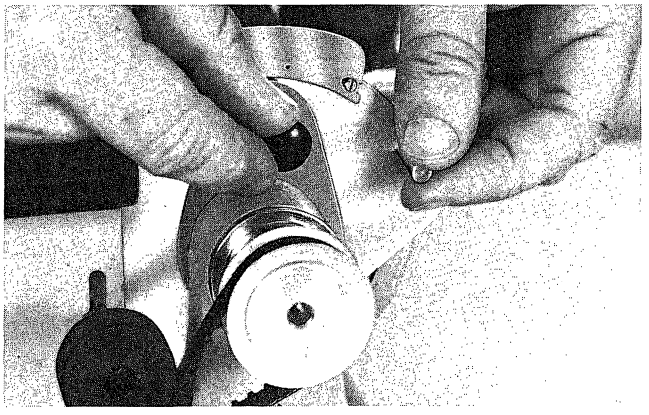


Figure 15

FAST FEED CONTROL

To quickly advance the specimen into cutting position, a fast feed control is provided. To use, turn the knurled knob counterclockwise as shown in Figure 14.

THICKNESS INDICATOR

The microtome can be quickly set for desired thickness of specimen section. The scale reads from 0 to 40 microns and is divided in 2 micron increments.

1. Loosen the black set screw knob on the back side of the thickness scale as shown in Figure 15.

2. With the left hand on the metal positioning shaft, as shown in Figures 15 and 16, move the scale until the arrow points to the desired thickness and turn set screw knob clockwise for locking action.

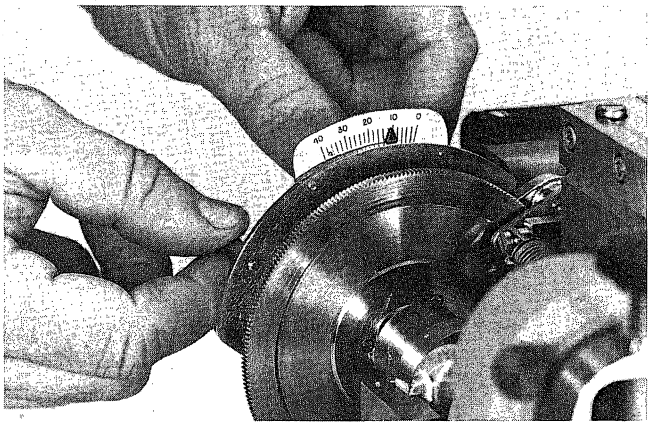


Figure 16

NOTE: Some technicians also use set screw knob for scale positioning to make the entire procedure a one-hand operation. The exact method used is strictly a matter of individual preference.

MATERIAL PREPARATION

Except in the case of very small specimens, the material can be squared off on the object disc. Material that has been squared off on the four outside edges can be sectioned with less tearing or loss of material.

The left side of the cabinet contains a large aluminum quick-freeze shelf frequently called a "heat sink", Figure 17. The heat sink has a high thermal conductivity and can draw the heat from a section very rapidly, because it is a very large mass of cold metal. Frozen material can be stored on object discs in the heat sink so that they are cold and ready for sectioning when needed, however they should be covered to prevent dehydration. The heat sink can also be used to freeze the specimen in approximately 30 to 60 seconds. Start with a cold object disc previously stored in the heat sink, put a few drops of blood serum, saline, or water on the object disc, and place the tissue on top of the liquid. To speed the freezing process, to approximately 10 seconds, the quick-freeze chamber which utilizes CO_2 may be used. See Figure 18.

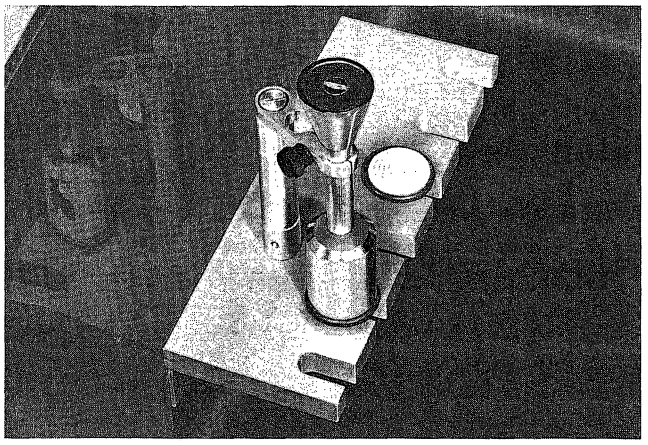


Figure 17

TEMPERATURE CONTROL

There is no one temperature which is best for freezing all types of tissue. Optimum temperature and rapidity of freezing varies with type of tissue being cut. The following table of suggested temperatures is furnished by courtesy of James B. McCormick M.D., pathologist, Swedish Covenant Hospital, Chicago.

OPTIMUM SPECIMEN FREEZING TEMPERATURES

Brain Lymph Node Thyroid Liver Kidney Spleen Uterine Currettings Testes Bladder Papilloma Bone Marrow Soft Parenchymatous Lesion	Zone I 0 to -15°C	Cryostat heat sink adequate to Zone I & II
Skin Without Fat Tongue Breast Without Fat Muscle Intestine Pancreas Prostate Cervix Uterus Ovary Adrenal	Zone II -15 to -30°C	
Skin With Fat Breast With Fat Adipose Omentum Industrial Waxes Cheese Butter Soft Rubber	Zone III -30 to -60°C	*Must use CO_2 freeze attachment, dry ice or liquid N_2

*See AO Reichert No. 834 Quick Freeze Chamber.

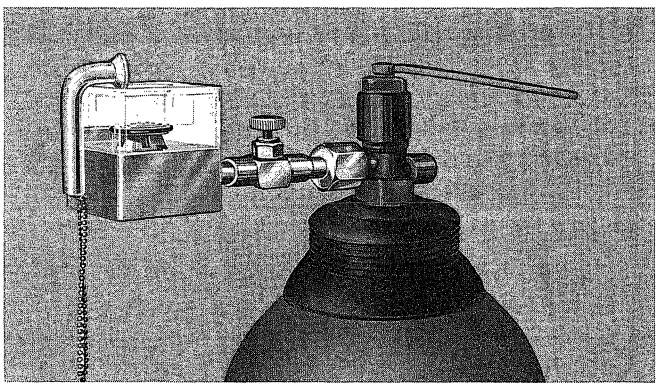


Figure 18. Quick-Freeze Chamber (Catalog No. 834)

REMOVAL OF CUT SECTIONS

One way to remove the section from the knife is to bring a warm slide or cover glass directly in contact with the section. Gradually lower the warm microscope slide onto the section. The cut section will immediately attach itself to the slide and remain in this position. The slide is now ready for processing. After picking up the cut section, a frost outline will appear on the knife. A Q-Tip swab is recommended for cleaning the frost residue from the knife. Cleaning the back side of the knife periodically is recommended as a buildup of residue on this back surface can be detrimental to sectioning.

FEED-SCREW MECHANISM

It will be noted that after a period of cutting, the feed-screw mechanism will reach the forward end of its travel. This will be indicated to the operator by failure of the handwheel to feed. Restore by turning the fast feed knob clockwise.

USING THE 840 MICROTOME FOR PARAFFIN SECTIONING

If there are extended periods of time when the HISTOSTAT Microtome is not being used, the microtome can be removed from the cabinet and used for paraffin sectioning. To adapt the microtome for such use, proceed as follows:

1. Remove the knife holder from the microtome and remove the microtome from the cabinet following the procedure in Steps 5 & 6 under "Cleaning Cabinet".

2. Thoroughly wipe knife, knife holder and microtome dry.

3. Remove the drive coupling (D, Figure 6) from the microtome by removing the large, slotted screw on the inside diameter of the coupling.

4. Remove handwheel from cabinet in same manner as coupling was removed and install handwheel on the microtome.

5. Lock anti-roll guide in upper position, Figure 12, or remove from holder.

CARE & MAINTENANCE

CLEANING CABINET

Periodically, depending upon usage, the HISTOSTAT cabinet should be thoroughly cleaned.

1. Turn the "Cool" switch to the "Off" position. Open cabinet door fully to allow unit to warm. While the cabinet is warming, proceed immediately with Steps 2 through 5.

CAUTION: Exercise reasonable care in handling cold metallic components of microtome.

2. Remove knife from knife holder and hold under warm running water for several minutes until knife reaches room temperature. Wipe dry and apply a light coat of oil before storing in box.

3. Brush any loose tissue from the base of the microtome into the bottom of the cabinet.

4. Remove the knife holder, brush and catch tray from the microtome.

5. Rotate the handwheel so that the microtome object clamp moves down to the low position.

6. To remove the microtome from the cabinet, take out the holddown bolt (watch that microtome does not tip backwards); shift the microtome to the left to disengage drive coupling from drive hub; and carefully lift out. You may find it more convenient to remove the glass door completely while removing the microtome.

7. When the microtome has reached room temperature, it should be thoroughly cleaned. Care should be taken that all moisture is removed from the slide-ways (See Figure 6) before the microtome is returned to the cabinet. Re-lubricate microtome in accordance with lubrication instructions.

8. Pull out the rubber drain plug in the bottom of the cabinet, Figure 3, and unscrew outside drain cap.

9. When the cabinet has reached room temperature, clean inside surfaces with soap and water. Rinse out cabinet; allow to drain and wipe dry with soft cloth.

10. Reinstall the microtome in accordance with instructions on page 3. Make sure the desired temperature has been set on the temperature control. Before restarting unit, be certain that the rubber plug and outside drain cap have been replaced.

GLASS DOOR REMOVAL AND MAINTENANCE

Pull the door to the fully closed position. Lift the front of the door above the rail, then move sufficiently forward to disconnect the connectors at the far edge of the door.

Use mild detergents or commercially available window cleaning solutions to clean glass surfaces.

Lubricate the door tracks as required. Use AO Catlog No. 8028 Spray or other quality silicone or TFE sprays.

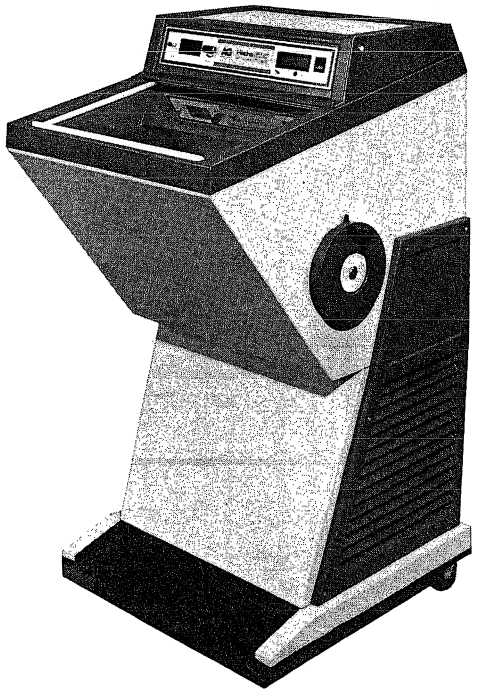


Figure 19

MICROTOME LUBRICATION

1. Lubricate the crank shaft at the oil cup, C in Figure 6, once per week or as needed.

2. Generously lubricate both the vertical and horizontal slides, B and E in Figure 6, using the special lubricant provided. Do not use substitute lubricants.

   AT THE END OF EACH WORKING DAY, CLEAN MICROTOME AND LUBRICATE THE SLIDES!

   ADHERENCE TO LUBRICATION SPECIFICATIONS IS IMPERATIVE. LACK OF LUBRICATION CAUSES SEIZURE OF THE SLIDES, RENDERING THE MICROTOME INOPERATIVE.

CLEANING CABINET CONDENSER

Periodically, depending upon room conditions, the face of the fin tubing of the cabinet condenser should be brushed clean of accumulated dust or other foreign material. A soft brush must be used and care taken not to damage fins.

BEFORE CLEANING THE CONDENSER, PUT "COOL" SWITCH TO "OFF" POSITION, AND UNPLUG THE HISTOSTAT.

To gain access to the condenser, remove the left side louver (when facing front of unit) which is held in place by six screws.

GENERAL COMMENTS ON "TECHNIQUE"

Proficiency

No amount of technique will overcome the handicap of a dull knife in the HISTOSTAT Microtome. The knife edge should be examined at approximately 100X magnification for nicks and resharpened as necessary. (For additional data on knives, request AO Reichert Manual 820-301).

Cleanliness of knife and anti-roll guide plate is of the utmost importance. Tissue clinging to either will result in torn sections. Frost also will produce the same results. The knife and anti-roll guide plate should be thoroughly cleaned using a "Q-Tip" and absolute alcohol and wiped dry. The cabinet should then be closed for a few minutes to allow the temperature to stabilize, or spray knife with Freon.

Brushes or "Q-Tips" used in the cabinet should be discarded if they are inadvertently exposed to room temperatures. Only a few degrees of heat will melt tissues which may be clinging to these items and using them on the knife will deposit grease which necessitates recleaning.

Check the back side of the knife frequently since tissue and mounting media have a tendency to build up and cause compression of the specimen.

The specimen should be trimmed before positioning the anti-roll guide plate at the edge of the knife. After trimming, bring the plate forward gradually by slowly turning knob A, Figure 12. The first few sections will roll up on the knife, but as the plate is fed forward you will observe that the sections slide between the plate and knife. The knife and plate should be very carefully cleaned of all these discarded sections. The plate position is critical and a few degrees turn on knob "A" will assure optimum results. A good light is very essential while sectioning.

The successful use of HISTOSTAT Microtome is largely a matter of technique. Mechanical adjustments have been kept to a minimum and results will depend on practice and patience. Many factors will affect sectioning including temperature, speed and method of freezing, the type of tissue and the condition of knife edge. Some tissues cut best at a relatively fast speed while others respond best to a slow uniform speed.

Cutting temperature can be very critical. When you have frozen a specimen using CO_2 or other quick-cold method (Freon, liquid N_2 etc.) sectioning cannot be done until the specimen temperature stabilizes at the setting of your cabinet. If the section crumbles it is likely that it is still too cold. For example, kidney fast frozen at -50°C with CO_2 will crumble until it warms up to between -15°C and -20°C.

As a rule of thumb, with a sharp knife the tissues listed in Zone I and II of the Temperature Chart can be cut to the following thickness for block sizes indicated.

4m/m sq.	2-4 microns
10m/m sq.	4-6 microns
20m/m sq.	10-14 microns

SERVICE

Complete repair facilities for microtomes are available at many AO Reichert authorized dealers and AO Reichert Technical Service Centers in Rosemont, IL, Chatsworth, CA, Edison, NJ, Dallas, TX, and Buffalo, NY. For cabinet repairs, contact your dealer for instruction.